



To the Chairman of the Scientific Jury
appointed by Order No 521/15.12.2022 by the
Director of NCIPD on the announced
competition for "Associate Professor" in the
higher education field 01.06.12
"Microbiology" at NCIPD
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REVIEW

For a competition for an academic position Associate Professor in the field of higher education 4 "Natural Sciences, Mathematics, Informatics" in the professional field 4.3. "Microbiology" for the needs of the Microbiology Department at NCIPD

With only candidate Chief Assistant Iva Petrova Trifonova, MD, Head of NRL "Vector-borne infections, listeria and leptospores" at NCIPD

By Prof. Dr. Todor Kantardjiev, MD, DSc

I have no general publications and no conflict of interest of any other nature within the meaning of para 1, items 3 and 5 of the Law for the Development of the Academic Staff in the Republic of Bulgaria with the candidate

All submitted and classified documents of the candidate for the academic position are accurately presented according to the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the NCIPD Rules and Criteria for the academic position "Associate Professor". According to the presented and supported by documents of Chief Assistant Iva Trifonova, MD, it definitely meets the minimum requirements (400 points) and exceeds them almost double! with 795 points for this competition.

1. Career development and biographical data

I have followed from the closely motivated and upward career development of the candidate, as Head of the Department of Microbiology and until two years ago, Director of the NCIPD.

Iva Trifonova acquired a bachelor's degree in molecular biology at Sofia University "St. Kliment Ohridski" in 2005, immediately after that she acquired a master's degree in molecular virology at the same faculty in 2007. From 2008 to 2010 is a PhD student at the NCIPD,

Department of Microbiology, NRL " Vector-borne infections, listeria and leptospire " with a scientific supervisor Prof. Iva Christova on the topic "Optimization of serological diagnostics and studies on the pathogenesis of Lyme borreliosis with recombinant antigens from *Borrelia burgdorferi*". In 2011 she acquires the educational and scientific degree "Doctor of Microbiology" at NCIPD. Her doctoral work impressed the scientific community with its innovation and relevance. Since 2017 she acquired a specialty in Microbiology (Laboratory Microbiology) and from 2021 started a specialization in the Laboratory Virology program. She worked consecutively as a biologist (2010-2012) at the NCIPD, and chief assistant (2012-2023). She is a Head of Laboratory NRL " Vector-borne infections, listeria and leptospire " at NCIPD, Department of Microbiology since 2020. At all colloquia for doctoral degree and for acquiring a specialty she has demonstrated in-depth knowledge, the result of systematic self-training. Her supervisor and NCIPD have provided her with excellent opportunities for qualification and self-improvement, as evidenced by specializations and courses abroad: She specialized at the Aristotle University of Thessaloniki, in Thessaloniki, Greece in 2010 and at the Istituto Superiore di Sanita in Rome, Italy in 2017, as well as taking part in courses, seminars and webinars at home and abroad, among them in Sweden in 2016, in Italy in 2017, in Denmark in 2018 and in three webinars of the Emerging Viral Diseases-Expert Laboratory Network (EVD-LabNet) in 2022.

2. Objects of research activity

Some of the scientific production of the candidate is in the current field of infectious diseases, which is of great epidemiological, clinical and therapeutic importance for our country and world science. Topics of scientific research are the causative agents and vectors of viral and bacterial transmissible infections, as well as some other medically significant bacteria and viruses.

The studies cover:

- viral causative agents of transmissible infections such as West Nile virus, Crimean-Congo haemorrhagic fever, yellow fever virus;
- bacterial causative agents of transmissible infections: *Borrelia burgdorferi* sensu stricto, *Borrelia burgdorferi* sensu lato complex, *Anaplasma* spp.;
- other bacteria such as *Leptospira* and *Listeria* spp,
- other viruses – *Dobrava-Belgrade* hantavirus, *Puumala* hantavirus, causative agent of COVID-19 pandemic SARS-CoV-2 etc.

Numerous research methods have been used such as:

- complement binding reaction, immunoblot and ELISA for IgM and IgG antibodies, serotyping
- conventional PCR, nested PCR, PCR c reverse transcription, Real Time PCR,
- sequencing of PCR products, phylogenetic analysis and wholegenome sequencing of West Nile virus.

ELISA tests with recombinant OspC, FlaB, OspA and VlsE protein antigens of *B. burgdorferi* have been developed and studied for the first time in Bulgaria and their high specificity and sensitivity and better accuracy than the ELISA tests with whole cell antigen have been demonstrated.

A cloning and expression system for immunodominant proteins of *B. burgdorferi* and original PCR techniques for the genes and plasmids of bacteria were approbated.

Large-scale studies were performed on birds and animals as reservoirs of the causative agents of Lyme borreliosis and Human granulocyte anaplasmosis by PCR and ELISA for antibodies, of *Dobrava* hantavirus (DOBV) and *Puumala* hantavirus with PCR, sequencing and clustering of *Leptospira* isolates with PCR. The incidence in vectors carrying infections - Crimean-Congo hemorrhagic fever in ticks and West Nile virus in mosquitoes - also was studied.

In addition to the above, other important studies are the complex studies of materials from patients with West Nile fever and Crimean-Congo haemorrhagic fever, of hantavirus infections in patients with haemorrhagic fever with renal syndrome (HFRS) and bacterial and viral infections in patients with vague febrile conditions, which etiologically illuminate the clinical aspect of these life-threatening infections of great importance for public health and the security of society.

3. Scientific production

The candidate Chief Assistant Iva Trifonova, MD participates in this competition with a total of 48 scientific papers, as journal articles in peer-reviewed scientific journals with an academic editorial board as follows:

- 22 publications in scientific journals with an international recognition and year-indexed impact factor. Almost all (21 articles) are after the PhD thesis
- participates in writing a chapter of a book on a scientific topic in 2022;
- 25 publications in our peer-reviewed scientific journals with an academic editorial board, 15 of these journal articles are after the dissertation.

Of all the scientific papers submitted, a total of 37 have been published since the dissertation. A large number of difficult to cultivate microorganisms have been studied, such as causative agents of Lyme borreliosis, human granulocyte anaplasmosis, hantaviruses and the many causative agents of other not yet well-studied worldwide viral transmissible infections. There are also studies on the causative agent of COVID-19, which shows a rapid reaction to newly emerging etiological factors. In Iva Trifonova's publications, both the methodological diversity and the precise definition of the topic concerning the latest scientific issues are impressive.

It is impressive that all 21 publications submitted after the dissertation are publications in journals with impact factor, the overwhelming majority of which are international. The candidate is the first author of 6 of the submitted publications, and all others show his methodical presence. He participated as a co-author of a chapter of the book on covid-19: "Viral load, dynamics of specific antibodies and levels of major cytokines depending on the severity of COVID-19".

The documented participation of Chief Assistant Iva Trifonova, MD, PhD in international scientific congresses and scientific conferences is a total of 27 pieces, of which 19 are after the defense of the dissertation, which is certified in the published collections with an academic editorial board. During the years of her scientific career at NCIPD she took part in almost all congresses and conferences on infections in Bulgaria (a total of 80, of which 63 after the defense of the dissertation). The presented evidences are also an indisputable indicator of her research activity and earned authority among the scientific community.

Chief Assistant Iva Trifonova, participates in research projects/programs, of which 4 are internationally funded and 5 are funded by the National Science Fund and one national program. A total of 10 scientific participations, which is important for creating scientific

contacts and increasing the expertise of the candidate. The publication activity shows the methodological guidance and support of prof. Iva Christova, DSc

4. Scientific indicators of scientific production

The total impact factor (IF) of the journal articles of Ch. Assistant Iva Trifonova is over 35, this strongly confirms significance and interest of the international academic community to them.

The article with the highest IF was published by the CDC's Emerging Infectious Diseases in 2016 with an impact factor of 6,751. Other journals that accepted her articles were as follows: Vector-Borne and Zoonotic Diseases in 2013, Journal of Medical Virology, two papers in 2015 and 2022, Ticks and Tick-borne Diseases in 2016, Infection, Genetics and Evolution in 2019, Journal of Infection and Public Health in 2020, and Journal of Clinical Virology in 2020.

The citations of the articles exceed about three times (146) the minimum required points (50) in the competition for associate professor, according to the officially accepted scientific criteria. Some of the citations are in many authoritative journals with very high IF (Frontiers in immunology, Eurosurveillance, Frontiers in Microbiology, Emerging Microbes & Infections). This is one of the most eloquent proofs of the high scientific value of the candidate's production.

5. Scientific contributions

In the scientific works of Chief Assistant Iva Trifonova are reflected the results of many years of systematic studies of the etiology, vectors and reservoirs of major vector-transmissible bacterial and viral zoonoses in our country, which are of importance for public health. Almost all are pioneers for the country and are associated with the introduction of a number of new research methods - different variants of Polymerase chain reaction (Real time RT-PCR, nested PCR) as well as genome sequencing of some viruses.

The contributions are grouped under the following main headings:

Environmental and epidemiological studies

Studies on rodents (articles Nos 2, 5, 6, 14, 20, 30, 43).

1. For the first time, *Dobrava* hantavirus (DOBV) infection was detected by the PCR method in different species of mice – *Apodemus flavicollis* (7.7%) and *Apodemus agrarius* (1.43%) in three regions of the country – Pazardzhik, Sliven and Smolyan. The S- and L- segments of the virus genome were partially sequenced and clustering of the Bulgarian isolates was made. Another study of rodents of the species *Apodemus agrarius*, *Apodemus sylvaticus*, *Apodemus flavicollis*, *Mus musculus*, *Microtus arvalis*, *Rattus rattus* and *Crocidura suaveolens* from regions from all areas in Southern Bulgaria with TaqMan Real time RT-PCR developed to detect hantaviruses in rodents reports that hantaviruses are founded mainly in the spleen and kidney and to a lower extent in the blood and lung of rodents. In total, specific hantavirus RNA was detected in 9 rodents. Eight of them were found in the area of the city. Peshtera, Pazardzhik region. One infected specimen has been proven in the area of the city. Sliven.
2. Establishment of the range of susceptible reservoirs for the causative agents of tick-transmitted infections Lyme borreliosis and Human granulocyte anaplasmosis. By applying polymerase-chain reaction for the first time in these ranges, the causative agents of Lyme

borreliosis in the species *Apodemus flavicollis*, *Apodemus agrarius* and *Apodemus sylvaticus* were found. As the highest percentage of infectivity is found in *A. flavicollis*.

3. Detection of specific antibodies with ELISA method. By PCR examination, the presence of *A. phagocytophilum* DNA was detected in *A. flavicollis*, *A. agrarius* and *A. sylvaticus*, with the highest infectivity being found in *A. agrarius*.

4. For the first time with molecular genetic methods, the circulation of Puumala hantavirus (PUUV) in the country has been proven. The virus was detected in *Myodes glareolus* (forest vole) in three regions of the country – Smolyan, Batak and Velingrad. A phylogenetic analysis was made of six sequences of the L-segment of the virus genome. Affiliation to the Alpine-Adriatic genetic line of PUUV has been established.

A first for the geographic range study of rodents for infestation with pathogenic leptospires was carried out. By nested PCR method *Leptospira* spp. DNA was detected in 28.44% (31/109) of the rodent samples of the species *Apodemus* spp., *Myodes glareolus*, *Microtus arvalis*, *Microtus* spp. (order Rodentia) and *Sorex minutus* (order Soricomorpha) from three districts of the country Pazardzhik, Smolyan and Plovdiv.

Pioneering for the country studies on vectors of viral and bacterial transmissible infections (articles Nos 7, 9, 17)

5. The presence of West Nile fever virus in *Culex pipiens* mosquitoes from the Pleven and Ruse regions has been proven. Wholegenome sequencing was conducted and affiliation to the Hungarian group of WNV genetic lineage 2 was confirmed. Comparative analysis of the sequence by a patient and by the vectors shows that they belong to different clusters.

A PCR study was carried out on ticks of the species *Hyalomma marginatum* and *Rhipicephalus sanguineus* for the presence of infection with Crimean-Congo hemorrhagic fever (CCHF) virus. A relatively high percentage (6.3%) of infection is found in *H. marginatum*, collected in the region of Burgas and Kardzhali. Affiliation to genetic line Europe 1 has been established. In the ticks of the species *R. sanguineus* s.l. (in 11.8% of the tested ticks), for the first time the presence of strain AP92, genetic line 2 in Bulgaria, was found, with the highest infection rate being found in the regions of Kardzhali and Haskovo.

Studies on viral transmissible infections (Articles Nos 8, 15, 17, 40, 39)

7. The circulation of West Nile virus gene line 2 in the country has been proven. A case of neuroinvasive ZNT has been confirmed molecularly. The genome of the virus was first sequenced and affiliation to the central/southern branch of genetic line 2 determined. For the first time in the country are introduced conventional and Real-time RT-PCR systems for detection of West Nile virus (lineages 1 and 2) (LAN), tick encephalitis (KE), Zika virus, Dengue viral fever, yellow fever, Tuscany virus, Usutu virus and Chikungunya. The following detection targets were selected: WNV - 5'-UTR (untranslated region) and part of the capsid gene; ZIKV- envelope gene; TBEV - 3' noncoding region; DENV - 5'-UTR (untranslated region); YFV - 5'-UTR (untranslated region); TOSV - the N-gene (S-segment); USUV - NS5 gene; CHIKV - nSP1 genes

Studies on viral haemorrhagic fevers (Articles Nos 10, 25, 26, 31)

9. The etiology of acute unclear febrile states has been studied to specify the role of hantaviruses and CCHF virus. Specific IgM antibodies against Hantaviruses were detected in 2.8% of the study group. The highest share of hantavirus infections was found in the districts of Burgas and Plovdiv. Slightly higher is the share (3.8%) of CCHF. The districts with the highest number of positive samples are Burgas and Sliven, followed by Plovdiv and Ruse.

The diagnostic capabilities of various serological tests for the detection of antibodies against hantaviruses - ELISA, complement fixation reaction (RSK) and immunoblot have been studied. The higher sensitivity and specificity of the ELISA and immunoblot method compared to complement fixation was confirmed.

11. The areas of expansion of the spread of the TSTB virus in Bulgaria are described. For the first time, the participation of PUUV Hantavirus as a cause of diseases in the country was confirmed and a comparison of epidemiological features and clinical manifestation in patients with DOBV and PUUV infection was made.

Pioneering for the country seroepidemiological studies on the spread of viral hemorrhagic fevers and vector-transmissible infections in the country (articles No. 9, 11, 12, 13, 16, 22, 38, 41, 44)

12. Several large-scale seroepidemiological studies have been made on the prevalence of CCHF, HFRS, WNF and TBE, covering all areas of the country. Both humans and domestic animals – cattle, goats and sheep were studied. The main areas of prevalence (highest seroprevalence) of the respective infections have been clarified.

13. The highest seroprevalence was found for *CCHFV* in the districts of Haskovo and Yambol.

14. The highest levels of seroprevalence of WNV were found in Sofia District (10%) and Vidin (7.5%), followed by Ruse and Silistra with 6% each. Overall seroprevalence for the country is relatively low (1.2%) and remains in the following years (1.5%). Very low levels of TBEV seroprevalation were found.

15. Seroprevalence levels of 24.4% of specific antibodies against Toskana virus, the causative agent of papatacy fever, were established, which for the first time confirmed the circulation of the virus in the country. The districts of Blagoevgrad, Kardzhali, Yambol, Varna and Pleven were identified as risk of infection with the highest seroprevalence.

16. A high seroprevalence (72%) of specific antibodies against CCHT virus in domestic ruminants was found. The highest seroprevalence was found in the district of Kardzhali (86.7% of the domestic animals studied), followed by the districts of Blagoevgrad, Burgas, Yambol and Haskovo.

17. For the first time in the country a serological survey of local and migratory wild birds was conducted for the causative agents of Lyme borreliosis and West Nile fever. The importance of *Turdus merula* and *Parus major* species as reservoirs of infections has been confirmed.

18. An overall low seroprevalence of TBE in the country (0.42%) was found among domestic ruminants. Specific IgG antibodies were found in 4 animals (cattle) from two districts of the country Pernik (6.67%) and Razgrad (6.25%).

Studies on leptospirosis in Bulgaria (articles Nos 27,32, 35, 36)

19. The capabilities of the reference microscopic agglutination test (MAT) and the ELISA test for the diagnosis of leptospirosis are compared. The results obtained show that the ELISA test is well applicable to the examination of samples taken at the beginning of the disease. In sera taken during the reconvalescent period, the sensitivity of the test is reduced. An analysis of the confirmed cases of leptospirosis was made. The highest share of cases is in the districts of Pazardzhik, Sofia, Montana, Shumen and Burgas. The leading serogroups causing leptospirosis in the country were *Leptospira icterohaemorrhagiae* (66.07%) and *Leptospira pomona* (19.64%).

Listeriosis serotyping (46)

20. Serotyping of *Listeria monocytogenes* isolates from clinical materials was made. There were 2 serotypes causing infections in the country - *Listeria monocytogenes* I serogroup 1/2a and *Listeria monocytogenes* II serogroup 4b.

First in the country studies on the antibody response against synthetic peptides in Lyme borreliosis (3)

21. The antibody response in patients with different manifestations of Lyme disease to four synthetic C6 peptide antigens (immunodominant areas IR6 of the VlsE surface protein of *B. burgdorferi* s.s. strains B31 and 297, *B. afzelii* (strain PT7) and *B. garinii* (IP90) by their application as antigens in ELISA.

22. C6 peptides from *B. burgdorferi* sensu stricto were found to have a higher reactivity than *B. afzelii* and *B. garinii* in patients with early manifestation of Lyme borreliosis (Erythema migrans as well as in Lyme arthritis (disseminated Lyme borreliosis). The C6 peptide from *B. garinii* has a higher reactivity in neuroborreliosis.

Pioneering for the country studies on viral load in patients with COVID-19 infection (19, 21, 23)

23. The viral load was found to be inversely dependent on the serum antibody response. The peak of the viral load is reached in the first days of the clinical manifestation of COVID-19. In patients with a more severe course of the disease, the viral load shows a slower decrease and is found for a longer time compared to patients with milder forms of the disease. Similarly, specific antibodies reach higher levels and persist for longer periods of time in patients with more severe clinical forms. Severe clinical forms correlate with advanced age, higher viral load, higher levels of IgA-antibodies and the cytokines IL-6, IL-10 and IL-18.

Studies of patients with vague febrile conditions and unspecified meningitis (37).

24. A study was made on serum samples from patients with unspecified viral meningitis, encephalitis and meningoencephalitis to clarify the etiological structure of the causative agents of acute CNS infections. Of the samples examined, 19.1% showed IgM reactivity. EBV, Coxsackie B viruses and influenza viruses (10%) are the most common causative agents followed by HSV-2, TBEV, VZV, and parainfluenza viruses (5.5%).

Contributions from dissertation (28, 29, 33).

Scientific-theoretical contributions: For the first time in our country systems for efficient cloning and expression of the main immunodominant proteins of *B. burgdorferi* – OspC, FlaB, OspA and VlsE have been developed;

Contributions of an applied nature: Original polymerase-chain systems have been developed to amplify the whole genes encoding the OspC, FlaB, OspA and VlsE proteins that can be applied to the genome demonstration of *B. Burgdorferi*;

Scientifically applied contributions: The first ELISA tests in Bulgaria with the recombinant OspC, FlaB, OspA and VlsE protein antigens have been developed.

6. Teaching activities

Chief Assistant Iva Trifonova also takes an active part in the training and teaching activities of the Microbiology Department of NCIPD. She is a sought-after teacher in various courses incl.

in postgraduate training courses. Responsively presents himself as a consultant in student training and in specialized courses.

7. Conclusion

The scientific contributions and high scientific indicators of Chief Assistant Iva Trifonova, MD fully meet the quantitative and qualitative criteria of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application and the criteria of the NCIPD for occupying the academic position of Associate Professor. The lecturing skills and high personal qualities of the candidate give me reason to support awarding the academic position of Associate Professor of Microbiology of Chief Assistant Iva Trifonova, MD, Head of NRL "Vector-borne infections, listeria and leptospines" of NCIPD and to recommend the same to the members of the honorable Scientific Jury.

Prof. Dr. T. Kantardjiev, MD, DSc